भारतीय मानक Indian Standard

तम्बाकू एवं तम्बाकू उत्पाद — तम्बाकू की परीक्षण पद्धतियाँ

IS 5643: 2019

(तीसरा पुनरीक्षण)

Tobacco and Tobacco Products — Methods of Test for Tobacco

(Third Revision)

ICS 65.160

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FOREWORD

This Indian Standard (Third Revision) was adopted by the Bureau of Indian Standards, after the draft finalized by the Tobacco and Tobacco Products Sectional Committee had been approved by the Food and Agriculture Division Council.

The methods of test prescribed in this standard are based on the methods followed by the manufacturers, consumers and testing authorities concerned in the country. This standard will ensure uniformity in test methodology for assessing the quality of tobacco products.

This standard was originally published in 1970 and subsequently revised in 1989 and 1999 and amended in 2005. In the second revision, provisions of conditioning of atmosphere as given in ISO 3402: 1991 'Tobacco and tobacco products — Atmosphere for conditioning and testing' were included and provisions of IS 7753: 1989 'Method for expression of analytical test results of tobacco (*first revision*)' were merged. Consequently, with the publication of second revision of this standard, IS 7753 was withdrawn. This revision has been undertaken to update various test methods in the standard in light of new scientific knowledge and advances in analytical techniques. The provisions of determination of total alkaloids by spectrometric method have been deleted in this revision in view of adoption of corresponding ISO Standard as Indian Standard under dual numbering system.

In reporting the result of a test or analysis made in accordance with this standard, is to be rounded off, it shall be done in accordance with IS 2: 1960 'Rules for rounding off numerical values (*revised*)'.

Indian Standard

TOBACCO AND TOBACCO PRODUCTS — METHODS OF TEST FOR TOBACCO

(Third Revision)

1 SCOPE

This standard prescribes the methods of test for tobacco.

2 REFERENCES

The standards given below contain provisions which, through reference in this text, constitute provisions of this standard. At the time of publication, the editions indicated were valid. All standards are subject to revision and parties to agreements based on this standard are encouraged to investigate the possibility of applying the most recent editions of these standards.

IS No.	Title
5194 : 1969	Method for determination of nitrogen — Kjeldahl method
9379 : 2008/ ISO 6488 : 2004	Tobacco and tobacco products — Determination of water content — Karl fischer method (third revision)
16121 : 2013/ ISO 3402 : 1999	Tobacco and tobacco products — Atmosphere for conditioning and testing
16026 : 2012/ ISO 15152 : 2003	Tobacco — Determination of the content of total alkaloids as nicotine — Continuous-flow analysis method
16132 : 2014/ ISO 2881 : 1992	Tobacco and tobacco products — Determination alkaloid content — Spectrometric method
16308 : 2016	Tobacco and tobacco products — Determination of water content — Gaschromatographic method

3 QUALITY OF REAGENTS

Unless specified otherwise, pure chemicals and distilled shall be employed in tests.

NOTE — 'Pure chemicals' shall mean chemicals that do not contain impurities which affect the experimental results.

4 SAMPLE PREPARATION

4.1 Draw sufficient amount of the test sample for the tests to be carried out. Keep aside 50 g of the sample

for the tests given under 6 and 7. If required, reduce the sample particle size to about 1 mm by grinding.

NOTES:

- 1 Grinding, cutting or undue handling of the sample can cause change in the moisture content of the sample. Moisture content should be carried out on sample as received as far as possible.
- 2 If the test sample has higher moisture than 15 percent, it cannot be ground and should be dried in air or at 40° C to reduce the moisture to about 10 percent or less.
- **3** Grinding can also cause changes in the sample due to heat generated. Hence, due care should be taken while grinding not to allow unnecessary high heat.

4.2 Storage

Sample as received and/or after particle size reduction and homogenization, should be packed in air tight containers and stored in conditions that will minimize deterioration and maintain sample integrity for any follow up analytical work. For long term storage, sample containers shall be stored below 4°C.

5 CONDITIONING AND TESTING ATMOSPHERE

The sample may be subjected to conditioning if, required by the provisions of the applicable standards or as agreed to between the buyer and seller. It is not applicable in the case of test methods for which particular test conditions are laid down elsewhere. The conditioning and testing atmospheres and duration of conditioning shall be as prescribed in IS 16121.

6 DETERMINATION OF OVEN MOISTURE

6.1 Principle

Tobacco loses mass on heating due to the loss of moisture and some volatiles. If the heating is done under strictly controlled set of parameters, this loss of mass is termed oven moisture in common tobacco terminology. This is also referred to as oven volatiles and loss on heating in some tobacco circles.

NOTE — For the determination of exact water in tobacco and tobacco products, *see* IS 9379 and IS 16308.

6.2 Apparatus

Usual laboratory apparatus and, in particular, the following:

6.2.1 *Dish*, made of aluminium, stainless steel, silica or porcelain and provided with a perforated cover. The

diameter of the dish shall be at least 50 mm and the depth not more than 40 mm.

- **6.2.2** Oven, fitted with a ventilator and means for forced internal circulation of air and maintained at a temperature of 100 ± 0.5 °C.
- **6.2.3** Analytical Balance, with a resolution of 0.0001 g.

6.3 Procedure

Place about 10 g of the material in a tared dish, close it with the perforated cover, weigh, and place it in the oven which shall previously have been brought to a temperature of 100 ± 0.5 °C. Maintain this temperature in the oven for 15 h. Remove the dish and allow it to cool in a desiccator. Weigh the dish with cover and the contents and note the weight of the material.

6.4 Calculation

Oven moisture, percent by mass =
$$\frac{100 (W_1 - W_2)}{W_1}$$

where,

 $W_1 = \text{mass in g, of the material taken for the test; and}$

 W_2 = mass in g, of the material after heating.

7 EXAMINATION FOR FREEDOM FROM WEEVILATTACK

7.1 Procedure

Take about 10 g of the material (*see* 4) on a large, clean sheet of paper. Loosen the lumps of the material and examine for the presence or absence of weevil (dead and alive) by naked eye (corrected for abnormal vision). A hand lens (magnification 10 X) may also be used. In case a larger magnification is used, this shall be stated in the test report.

8 DETERMINATION OF TOTAL NICOTINE ALKALOIDS AS NICOTINE

8.1 Principle

Determination of total nicotine alkaloids may be carried out either gravimetrically by silico-tungstic acid method (see 8.2), or by spectrophotometric method (see IS 16132), or by continuous flow analysis method (see IS 16026). Nicotine alkaloids complex with silico-tungstic acid in acidic medium to form insoluble nicotine silico-tungstate. The mass of the precipitate is determined by filtration using an ash less filter paper in combination with incineration or a sintered glass crucible in combination with oven-drying

8.2 Silica-Tungstic Acid Method

8.2.1 Apparatus

Normal laboratory apparatus in particular the following:

- **8.2.1.1** Kjeldahl flask, 500 ml capacity.
- **8.2.1.2** Distillation assembly The assembly apparatus consists of a distillation flask 500 ml (Kjeldahl flask or a round-bottom flask) of suitable capacity, having a provision for fitting a splash head and an inlet tube for steam reaching the bottom of the flask. The free outlet of the splash head is connected to a well cooled condenser. The delivery tube of which dips below the surface of dilute hydrochloric acid contained in a receiving Erlenmeyer flask. It should also be possible to heat the distillation flask, using a burner, heating mantle or some such heating device, to maintain a constant liquid level during distillation.
- **8.2.1.3** Crucible, silica or platinum for incineration.
- **8.2.1.4** Laboratory oven, capable of maintaining 120 ± 5 °C.
- **8.2.1.5** Sintered glass crucible Gooch type, of porosity 2 (40 μ m to 100 μ m).
- **8.2.1.6** Filter flank (Buchner flask.)
- 8.2.1.7 Vacuum source.
- **8.2.1.8** *Desiccator*, containing an effective drying agent.
- **8.2.1.9** *Analytical balance*, with a resolution of 0.0001 g.
- 8.2.1.10 Bunsen burner
- 8.2.2 Reagents
- 8.2.2.1 Liquid paraffin
- **8.2.2.2** *Sodium hydroxide solution*, aqueous 30 percent (m/v).
- 8.2.2.3 Sodium chloride
- **8.2.2.4** *Phenolphthalein indicator solution*, 0.2 *percent* (m/v) Dissolve 0.2 g of phenolphthalein in 60 ml of rectified spirit and dilute to 100 ml with water.
- **8.2.2.5** *Dilute hydrochloric acid*, 1 : 4 and 1 : 1 000 (v/v).
- **8.2.2.6** Silico-tungstic acid solution Dissolve 120 g of silico-tungstic acid (4H₂O, SiO₂, 12WO₃, 22H₂O) in 100 ml water and dilute to one litre. The solution should be free from cloudiness and green colour.

NOTE — The silico-tungstic acid should be white or pale yellow crystals free from green colour. Of the silico-tungstic acids, $4\mathrm{H}_2\mathrm{O}$, SiO_2 , $10\mathrm{WO}_3$, $3\mathrm{H}_2\mathrm{O}$, and $4\mathrm{H}_2\mathrm{O}$, SiO_2 , $12\mathrm{WO}_3$, $20\mathrm{H}_2\mathrm{O}$ should not be used as these do not give crystalline precipitate with nicotine.

8.2.2.7 *Methyl orange indicator solution*, 0.04 percent (m/v) in aqueous ethyl alcohol (20 percent by volume).

8.2.3 Procedure

8.2.3.1 Weigh accurately a quantity of the material that shall contain preferably a minimum of 0.1 to 1.0 g of nicotine in a suitable tared vessel. Quantitatively, transfer the weighed material to the distillation flask (a 500 ml Kjeldahl flask or a round bottom flask) using water to wash the last traces of the material into the flask. If necessary, add to the contents of Kjeldahl flask or a round bottom the distillation flask a small amount few drops of liquid paraffin to prevent frothing during distillation and a few small pieces of pumice stone to prevent bumping. Add 10 ml of sodium hydroxide solution (see 8.2.2.2) using and a few drops of phenolphthalein as indicator. Fit the mouth of the Kjeldahl flask with a two holed, rubber stopper through which pass the stem of a trap bulb and an inlet tube for steam. Connect the free end of the trap bulb to a condenser. Assemble the distillation unit with the receiving flask containing 10 ml of dilute hydrochloric acid (1:4), the delivery end of the condenser dipping well below the surface of the liquid in the receiving

Connect the steam inlet tube to the source of steam and pass steam in the distillation flask to start distillation. Distil rapidly with the current of steam. When the distillation is well under way, heat the distillation Kjeldahl flask using Bunsen burner to reduce the volume of contents of the flask as far as practicable without causing bumping or undue separation of insoluble matter externally to maintain constant liquid level in the flask during distillation. Continue the distillation until a small quantity of the distillate shows no cloudiness or opalescence when treated with a drop of silico-tungstic acid and a drop of dilute hydrochloric acid (1:4). Confirm the alkalinity of the residue in the distillation Kjeldahl flask with phenolphthalein indicator solution.

Reduce the volume of the distillate by concentrating it on a steam boiling water bath (see Note) and make up the volume of the concentrated distillate to a convenient volume in a graduated flask with water at room temperature. Mix the contents of the graduated flask thoroughly and filter through a dry filter paper if it is not clear. Collect the filtrate in a convenient flask. Test a portion of this filtrate with methyl orange indicator to confirm its acidity.

NOTE — By heating on a steam-boiling water bath, the nicotine content of the distillate is not affected.

8.2.3.2 Pipette an aliquot (*see* Note 1) of the filtrate, containing about 0.1 g of nicotine into a beaker and add at the rate of 3 ml of dilute hydrochloric acid (1 : 4) for each 100 ml of the aliquot and one ml of silico-tungstic acid solution for every 0.01 g of nicotine supposed to be present in the aliquot. Stir the contents of the beaker thoroughly, heat it on a water bath for about 30 min till the precipitate settles down and allow to stand overnight

in a refrigerator. Before filtering, stir the contents of the beaker to see that the precipitate settles down quickly and is in a crystalline form.

NOTES

1 If the nicotine content of the material is very low, an aliquot containing at least $0.0\,1$ g of nicotine should be used.

2 The mass of the precipitate can be determined by collecting the precipitate on a filter paper followed by incineration (see 8.2.4) or by collecting on a sintered glass crucible and drying (see 8.2.5).

8.2.4 Filter Paper Incineration Method

8.2.4.1 Filter the contents of the beaker through an ash less filter paper. Wash all the residue remaining in the beaker on to the filter using dilute hydrochloric acid (1:1000) at room temperature. Wash the filter paper with dilute hydrochloric acid (1:1000) until a few millilitres of the filtrate do not produce a precipitate or opalescence when tested with a few drops of the distillate containing nicotine.

8.2.4.2 Dry the crucible (*see* **8.2.1.3**) in the laboratory oven until it shows constant weight (\pm 1 mg). Store the crucible in a desiccator (*see* **8.2.1.8**).

8.2.4.3 Transfer the filter paper containing the precipitate to the tared crucible (*see* **8.2.4.2**). Place the crucible on a silica triangle resting on a tripod, heat gently at first to dry and then ignite the filter paper with a Bunsen burner completely to ash until all carbon is oxidized. Finally, heat the platinum crucible over a Bunsen burner for not more than 10 min. Cool the crucible in a desiccator (*see* **8.2.1.8**) and weigh. Repeat the heating process until a constant weight (\pm 1 mg) is obtained.

NOTE — If the nicotine content of the material is very low, an aliquot containing at least 0.0 1 g of nicotine should be used.

8.2.4. 4 Calculation

Total alkaloid as nicotine (on dry basis), percent by mass = $\frac{0.1141 (W_1 \times V_1)}{(W_2 \times V_2) (100 - M)}$

where.

 $W_1 = \text{mass in g, of the residue in the crucible;}$

 V_1 = total volume in ml, of the concentrated distillate after making up the volume (see 8.2.3.1);

 W_2 = mass in g, of the material taken for steam distillation;

 V_2 = volume in ml, of the aliquot of the filtrate taken for the precipitation (see **8.2.3.2**); and

M = moisture, percent by mass (see 6.4).

8.2.5 Sintered Glass Crucible Method

8.2.5.1 Dry the sintered glass filter crucible in a laboratory oven to constant mass (\pm 1 mg). Store in a desiccator. Weigh to the nearest 0.000 1 g, the dried sintered glass crucible and filter the precipitate directly

into the glass filter using a Buchner flask connected to a vacuum source. Ensure that all the precipitate is removed from the sides of the beaker and the glass stirring rod by rinsing three times with hydrochloric acid solution using 15 ml each time and transferring the rinse to the crucible. Discard the washings.

Rinse with a further aliquot of hydrochloric acid (up to 400 ml may be required) which should be collected and tested with a few drops of nicotine solution to ensure that no opalescence occurs that is, all silico-tungstic acid has been removed. Dry the sintered glass crucible with the precipitate in the laboratory oven, cool in the desiccator and weigh to the nearest 0.0001 g. Place the crucible back in the oven for 1 h, allow to cool and reweigh. Repeat until a constant weight ± 1 mg is obtained.

8.2.5.2 Calculation

Total alkaloid as nicotine (on dry basis), percent by mass = $\frac{0.\ 1012\ (W_1 \times V_1)}{(W_2 \times V_2)(100 - M)}$

where.

 $W_1 =$ mass in g, of the residue in the crucible;

 V_1 = total volume in ml, of the concentrated distillate after making up the volume (see 8.2.3.1);

 W_2 = mass in g, of the material taken for steam distillation;

 V_2 = volume in ml, of the aliquot of the filtrate taken for the precipitation (see **8.2.3.2**); and

M = moisture, percent by mass (see **6.4**).

8.3 Spectrophotometric Method

Total nicotine alkaloids can be determined by a spectrometric method prescribed in IS 16132.

8.4 Continuous Flow Analysis Method

Total nicotine alkaloids can also be determined by continuous flow analysis as prescribed in IS 16026.

8.5 Repeatability

Duplicate determination should agree within 0.05 percent by mass of nicotine. If not, further duplicate determinations should be made until this requirement is fulfilled.

9 DETERMINATION OF TOTAL NITROGEN

9.1 Principle

Total nitrogen is the amount of both organic and inorganic nitrogen present in the tobacco sample. The sample is first pre-digested with reducing agents to convert inorganic nitrogen (nitrate and nitrite) into ammonium salt followed by digesting with oxidizing agents to convert all nitrogenous matter into ammonium sulphate. Ammonia in the Kjeldhal digest is quantitatively liberated by steam distillation,

analyzed and expressed as total nitrogen. The ammonia concentration in the distillate can be determined using titrimetric, colorimetric or specific ion electrode determination methods. The method prescribed here employs the titrimetric method.

9.2 Apparatus

Usual laboratory apparatus and, in particular, the following:

9.2.1 Kjeldahl digestion flask, 500 ml or of suitable capacity.

9.2.2 Kjeldahl distillation apparatus with distillation flask of suitable capacity (*see* IS 5194)

NOTE — Kjeldahl apparatus from micro to macro scale and consisting of digestion and distillation in one unit are available commercially. These units work satisfactorily and can be used following the user instructions provided by the supplier.

9.3 Procedure

9.3.1 Digestion

Transfer about 1 g of sample (see 4) accurately weighed to 0.0001 g, in a 500 ml Kjeldahl digestion flask containing 1 g of salicylic acid and add 30 ml of concentrated sulphuric acid (see 9.3.1) rinsing the neck of the flask. Shake until thoroughly mixed and allow to stand, shaking frequently, for at least 30 min. Add 5 g of sodium thiosulphate and heat the solution for 5 min. Cool and add a mixture of 10 g potassium sulphate or anhydrous sodium sulphate and 1 g copper sulphate carefully to avoid sticking of the mixture in the neck of the flask. Heat very gently keeping the flask in an inclined position until foaming ceases. Increase the heat till acid boils briskly, continue boiling till the digest becomes clear and green and digest for a few more minutes (total digestion time is approximately two hours).

NOTES:

1 Readymade salt catalyst mixtures available commercially can also be used following the instructions provided by the supplier.

 ${\bf 2}$ This digesting operation shall be carried out in a fume hood .

9.3.2 Distillation

After cooling, dilute with 200 ml of water. Transfer the contents quantitatively to the distillation flask by rinsing the Kjeldhal flask repeatedly. Add a few pieces of granulated zinc or pumice stone to prevent bumping. Add a few drops of phenolphthalein indicator. Assemble the Kjeldahl distillation unit. Connect the receiving Erlenmeyer flask or beaker containing 50 ml of 4 percent boric acid solution and a few drops of 0.5 ml mixed indicator, ensuring that the delivery end of the condenser is below the level of the liquid in the receiving flask. Add gradually 100 ml of sodium hydroxide solution through the funnel to make the contents thoroughly alkaline as indicated by the phenolphthalein indicator. Heat the distillation flask

to boiling and distil about 150 ml of the distillate in the receiving flask. Lower the receiving flask to raise the delivery tube above the liquid, distil further for a few seconds. Rinse the tube with water into the flask. Remove the flask.

9.3.3 Titration

The content in the receiving flask is titrated with 0.1 N sulphuric acid (*see* **9.3.2**), the end point in case of the mixed indicator, being the colour turning from green to pink and in the case of Sher indicator from blue to pale pink/yellow.

9.3.4 Carry out a blank determination using all reagents in the same quantities but without the material to be tested.

9.4 Calculation

Total nitrogen, percent by mass (on dry basis) $= \frac{(V_1 - V_0) \times N \times 140}{W(100 - M)}$

where,

V_o = volume in ml, of standard 0.1 N sulphuric acid solution used to titrate the blank distillate;

 V_1 = volume in ml, of standard 0.1 N sulphuric acid solution used to titrate the sample distillate;

N = normality of standard sulphuric acid solution;

W =mass in g, of the material taken for the test; and

M = moisture, percent by mass (see 6.4).

10 DETERMINATION OF TOTAL ASH

10.1 Procedure

10.1.1 Accurately weigh about 10 g of the material (see 4) into a tared 9 cm diameter platinum, porcelain or silica dish. Carefully dry the material on a burner flame and char it completely until all organic matter is destroyed. Ignite the charred material by placing the dish in a muffle furnace maintained at a temperature of 550 ± 25 °C for 2 h. Cool the dish and weigh. Note the mass of the ash contained in the dish.

10.1.2 Preserve the ash for the determination of acid insoluble ash.

10.1.3 Calculation

Total ash content of the material (on dry basis), percent by mass = $\frac{10\ 000\ W_1}{W_1(100\ -\ M)}$

where,

 $W_1 = \text{mass in g, of the ash;}$

 $W_2 = \text{mass}$, in g, of the material taken for the test;

M = moisture content, percent by mass (see 6.4).

11 DETERMINATION OF ACID INSOLUBLE ASH (SILICATE RESIDUE INSOLUBLE IN HYDROCHLORIC ACID)

11.1 Reagents

11.1.1 *Dilute Hydrochloric Acid*, 1:1 (v/v).

11.1.2 Concentrated Nitric Acid, sp gr 1.42.

11.2 Procedure

11.2.1 Moisten the ash contained in the dish (see 10.1.2) with a few drops of water. Cover the dish and carefully add 20 ml of dilute hydrochloric acid avoiding loss due to effervescence. Place the covered dish on a water bath and digest for 20 to 30 min. Remove and rinse the cover, add one millilitre of concentrated nitric acid to oxidize any ferrous salts and evaporate the contents to dryness. Heat for about 30 min on water bath to dehydrate the silica. If necessary, heat for one hour in an oven at 110°C to complete the dehydration. Moisten the dry salt with 10 ml of dilute hydrochloric acid and 50 ml of water. Heat on water bath until all soluble salts are in solution. Filter through a filter paper (Whatman no. 44 or equivalent) and collect the filtrate in a 500 ml volumetric flask. Transfer all the residue to the filter paper and wash several times with hot dilute hydrochloric acid.

11.2.2 Transfer the filter paper along with the residue to a platinum dish or silica crucible and ignite to bright red heat. Cool and weigh the material.

11.3 Calculation

Acid insoluble ash (on dry basis), percent by mass $= \frac{10\ 000\ (W_2 - W)}{W_1(100 - M)}$

where,

W =mass in g, of the empty dish;

 W_1 = mass in g, of the material taken for the test (see 11.1.1);

 W_2 = mass in g, of dish with acid insoluble ash (see 11.2.2); and

M = moisture content, percent by mass (see 6.4).

12 DETERMINATION OF TOTAL CHLORIDES

12.1 Principle

Chloride reacts with silver nitrate to precipitate silver chloride stoichiometrically. The quantity of silver nitrate exhausted in the reaction is a direct measure of total chlorides present in the sample. The analysis can be done using potentiometric, iodometric titration or colorometry. The method prescribed here is based on potentiometric titration.

12.2 Apparatus

12.2.1 *pH* Meter, equipped with silver and glass electrodes.

12.2.2 *Burette*, 10 ml capacity, graduated in 0.05 or 0.02 ml, units.

12.3 Reagents

12.3.1 Standard Silver Nitrate Solution, 0.1 N — Standardize against potassium chloride as per the procedure prescribed in **12.4**.

12.3.2 *Dilute Nitric Acid*, 1 : 9 (v/v).

12.4 Procedure

Weigh accurately about 2 g of tobacco sample (*see* 4) into a 250 ml beaker. Add 100 ml of water, a small amount in the first instance to wet the tobacco thoroughly and then the remainder. Allow it to stand for at least 5 min at room temperature, stirring intermittently. Add 5 ml of dilute nitric acid into the mixture and insert the clean electrodes. Start magnetic stirrer and continue stirring throughout titration at a rate sufficient to produce vigorous agitation without sputtering. Titrate with standard silver nitrate solution to the

potential previously established as equivalent point. Determine equivalence point graphically by making several titrations on one or more tobacco samples. Recheck occasionally and determine when either electrode is replaced. Record the volume of the titrant.

12.5 Calculation

Total chlorides (on dry basis), percent by mass $= \frac{V \times N \times 354.533}{W(100 - M)}$

Where,

V = volume in ml, of silver nitrate solution required for the test;

N = normality of silver nitrate solution;

W =mass in g, of the sample taken for the test; and

M = percent moisture content (see **6.4**).

13 REPORTING

13.1 Basis of Expression of Results

The results of test according to any of the agreed methods shall be reported as a percentage of the moisture free mass of the sample.

Bureau of Indian Standards

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Review of Indian Standards

Amendments are issued to standards as the need arises on the basis of comments. Standards are also reviewed periodically; a standard along with amendments is reaffirmed when such review indicates that no changes are needed; if the review indicates that changes are needed, it is taken up for revision. Users of Indian Standards should ascertain that they are in possession of the latest amendments or edition by referring to the latest issue of 'BIS Catalogue' and 'Standards: Monthly Additions'.

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Amendments Issued Since Publication

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